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(54) Title: METHODS FOR SELECTING PROSTATE CANCER TREATMENTS AND TREATING PROSTATE CANCER

(57) Abstract: A method for selection treatment for or treating a patient afflicted with prostate cancer by determining whether the number of copies of HER-2/neu gene in prostate cells from the patient exceeds four by in-situ hybridization and aggressively treating such patient having prostate cells with five or more copies of the HER-2/neu gene. Aggressive treatments include radiation in the range of 60-75 Gy or an anti-HER-2/neu antibody alone or in combination with an anti-androgen.

METHODS FOR SELECTING PROSTATE CANCER TREATMENTS AND TREATING PROSTATE CANCER

This application is a continuation-in-part of U.S. Serial No. 09/152,934, filed September 14, 1998, which is a continuation-in-part of U.S. Serial No. 09/088,417, filed June 1, 1998, which is a continuation-in-part of U.S. Serial No. 08/832,745, filed April 4, 1997, the contents of which are hereby incorporated by reference in their entirety into the present application.

FIELD OF THE INVENTION

The present invention relates to determining treatment of and treating neoplastic disease, and more particularly treatment for prostate cancer.

BACKGROUND OF THE INVENTION

Prostate cancer is the most common cancer in American men and the second leading cause of cancer death. The disease is responsible for nearly 3% of all deaths in men over the age of 55 years and it is likely that more than 300,000 new cases of prostate cancer will be diagnosed in American men this year.

The ability to monitor neoplastic disease status is an important tool in prostate cancer therapy. In addition to improving prognostication, knowledge of the disease status allows an attending physician to select the most appropriate course of therapy.

Since severe patient distress can be caused by more aggressive therapy regiments, it is desirable to determine which patients require such aggressive therapies. For example, patients with a high likelihood of relapse can be treated aggressively with powerful systemic chemotherapy and/or radiation therapy. Where there is a lesser likelihood of relapse, less aggressive therapies can be chosen.

Traditional therapies for prostate cancer include observation, radiation therapy, and radical prostatectomy, with radiation being the definitive choice because of the high disease-specific survival rates. More recently, treatment with anti-androgens has been used and new cancer-specific therapeutic products are currently being developed, expanding the spectrum of potential treatments.

Prostate cancer arises in a tissue whose development and function is strongly dependent upon hormones. Not surprisingly, therefore, these tumors are frequently stimulated by hormones, in particular androgens which are steroid hormones of high potency such as testosterone. Removal or inhibition of androgens plays a major role in therapy of this malignancy for some patients and this characteristic has been used in the design of endocrine manipulative therapy for prostate cancer. Hormonal manipulation is design primarily to decrease serum androgens or their effects and has been reported to effect improvement in symptoms of up to 80% of patients with prostate carcinoma. The primary aim of such treatments is to deprive the prostatic tumor of trophic androgens. When effective, hormone manipulations cause tumor regression in two ways: (i) by removing hormones that directly stimulate tumor growth, and (ii) by blocking production or release of other trophic factors that are direct stimuli for tumor growth. Two potent anti-androgens, cyproterone acetate and flutamide, have been used to treat patients with prostatic cancer. These agents have multiple effects, but their principal action is to inhibit binding of testosterone or dihydrotestosterone to the androgen receptor.

Unfortunately, following hormonal treatment resistance to the drugs develops, particularly in metastatic disease or when diagnosed earlier in life, sometimes the tumors eventually regrow. In such a situation, the tumors are unresponsive to hormonal manipulation. Furthermore, many tumors that arise in hormone-responsive tissues do not respond to hormonal manipulations at all.

In current urologic practice, a subset of men often diagnosed early, before the age of 60, will either present with metastatic disease or relapse after primary surgery or radiation treatment. These men initially respond to anti-androgen therapy, but ultimately become insensitive to the treatment. It has been known for decades that most prostate cancer metastases will initially regress if they are deprived of androgen stimulation. Originally, anti-androgen therapy consisted either of bilateral orchiectomy (castration) or exogenous administration of diethyl stilbestrol (estrogens). In the last ten years, new anti-androgen drugs were developed that either block androgen receptor(s) or are lutenizing hormone releasing factor antagonists.

A small percentage of anti-androgen treated men are refractory to therapy at the onset. Most responded initially and if they were going to become hormone treatment resistant,

developed symptomatic metastases and/or rising serum PSA (prostate specific antigen) levels after months or years of hormone-based therapy. It is assumed that either new genetic mutations occur in the "dormant" metastatic sites that confers the ability for the tumor to grow again in the absence of androgen stimulation or that clones of hormone-independent tumor that were "masked" by the initially faster growing hormone dependent cells. The hormone-independent tumor cells are then permitted to grow without competition as these hormone dependent cells are suppressed by the anti-androgen therapy.

Accordingly, there is a clear need for new indicators of prostate cancer prognosis and for adjuvant or alternative therapeutic approaches to prostate tumors that grow independent of androgens and are therefore not fully responsive to hormonal manipulations.

Prostate cancer has variable clinical outcome and recent studies indicating the potential benefits of withholding therapy in older men with limited disease and the potential to predict inoperable cancer in men with aggressive tumors has prompted the search for new prognostic markers that would be applied to the initial guided prostate needle biopsy and prove successful in selecting therapy and predicting disease outcome. Since severe patient distress can be caused by more aggressive therapy regimens, it is desirable to determine when such therapies are warranted. For example, patients with a high likelihood of relapse can be treated aggressively with powerful systemic chemotherapy and/or radiation therapy. Where there is a lesser likelihood of relapse, less aggressive therapies can be chosen. It is also desirable to identify those patients who might be candidates for newly developed target-specific therapies such as those described herein. There is thus a clear need for new assays to predict which tumors would likely respond to particular treatment regimes such as the aforesaid adjuvant or alternative therapeutic approaches thereby allowing an attending physician to select the most appropriate course of therapy.

The identification of new prognostic markers in prostate cancer would allow urologists to stratify their patients into groups that could receive significantly different therapies. Tumor grade and DNA ploidy have been generally accepted as significant predictors of out come for the disease (see e. g., Ross et al., Cancer, 74: 2811-18 (1994). These indicators, however, are subject to limitations. For example, tumor grading the Gleason method can be inaccurate for intermediate grade tumors. This is due to the inability to capture the most representative

morphologic pattern of the lesion in a narrow-bore needle biopsy. A clearly established prognostic panel capable of defining therapy selection is highly desirable.

The HER-2/neu (c-erbB-2) gene is localized to chromosome 17q and encodes a transmembrane tyrosine kinase growth factor receptor with substantial homology to the epidermal growth factor receptor. HER-2/neu expression in breast cancer has generally been accepted as a predictor of disease outcome with HER-2/neu gene amplification by Southern analysis and corresponding over expression of HER-2/neu protein ($p185_{neu}$) by Western blotting or immunohistochemistry (IHC) predicting early disease relapse in lymph node negative and lymph node positive patients. See Battifora, et al., Modern Pathol,(1991) 4:466-474; Press, et al., Cancer Res, (1993) 53:4960-4970; Seshadri, et al, Clin Oncol, (1993) 11:1936-1942; Descotes, dt al., Anticancer Res, (1993) 13:119-124; Muss, et al., N Engl J Med, (1994) 330:1260-1266; Tetu, et al., Cancer, (1994) 73:2359-2365; Marks, et al., Annal Surg, (1994) 219:32-341. Recently, amplification of the HER-2/neu gene or over expression of the HER-2/neu protein have been clinically utilized to identify patients likely to be refractory to less intense cytotoxic chemotherapy in breast cancer. Muss, et al., supra. Moreover, clinical trials featuring patients with HER-2/neu protein have shown promise for the treatment of refractory metastic ovarian and breast cancer. See Baselga, et al., J Clin Oncol, 14(3):737-44 (1996); Peitras. et al., Oncogen, 9(7): 1829-1838 (1994).

In prostate cancer, a consensus as to the predictive value of HER-2/neu gene amplification and $p158_{neu}$ protein expression has not been reached. The majority of published prognostic studies of HER-2/neu status in prostate cancer utilized immuohistochemical techniques featuring a variety of antibodies with differing sensitivities and specificities particularly when utilized in archival specimens. See, e.g., Visakorpi , et al., Modern Pathol, (1992) 5:643-648; Ibrahim, et al.,Surg Oncol, (1992) 1:151-155; Ross,et al., Cancer, (1993) 72:3020-3028;; Sandavisan, et al., J Urol, (1993) 150:126-131; Kuhn, et al., J Urol, (1993) 150:1427-1433; Melon, et al., J Urol, (1992) 147:496-499. Molecular based studies of the HER-2/neu gene in prostate cancer have been limited to two published reports from one research group which reported an absence of gene amplification by Southern analysis in a small number of prostate cancer specimens, i.e., Latil, et al., Int J Cancer, (1994) 59:637-638; Fournier, et al., Urol Res, (1995) 22:343-347. In one report using the MAB-1 antibody, no staining could be identified on archival fixed tissue specimens. Visakorpt, et al., Modern

Path., supra. In another study, immunoreactivity for HER-2/neu oncoprotein was more intense in prostatic hyperplasia and prostatic intraepithelial neoplasia than in adenocarcinoma. Ibahim, et al., supra. Several previously published immunohistochemical studies of HER-2neu in prostate cancer have failed to link expression with disease outcome. In one study using the paB-1 antibody on formalin-fixed paraffin-embedded archival material, HER-2/neu oncoprotein expression was identified in one of the clinically localized prostate cancers, but did not appear to be a significant prognostic marker. See Kuhn, et al., supra. A significant decrease of EGF receptor and increased immunodetection of HER-2/neu protein was identified in prostate cancer but the findings did not correlate with tumor stage or grade. See Melon, et al., supra. Finally, in a more recent study of prostate cancer and benign prostatic hyperplasia using the AB-3 antibody on archival tissue, $p185_{neu}$ immunostaining did not correlate with Gleason grade and a trend toward an inverse relationship was presented. See Gu, et al., Cancer Letters, (1996) 99:185-189.

Several immunohistochemical studies of HER-2/neu protein expression in prostate cancer have correlated with other prognostic variables and suggested correlation with disease outcome. In one study using an immunoalkaline proshatase procedure and the 9G6 antibody, HER-2/neu protein expression was found in 16 of 100 (16%) of prostate cancer specimens and protein expression correlated with high tumor grade and aneuploid DNA content. See Ross, et al., supra. In another study utilizing the TA-1 antibody, over expression of HER-2/neu protein was found to be an indication of poor prognosis in prostate cancer and correlated with high histologic tumor grade, disease state and DNA aneuploidy. See Sandavisan, et al., supra. In a study featuring analysis of a group of potential prognostic markers, HER-2/neu antigenicity was found to be a predictor of prostate cancer progression on univariate analysis and also significantly contributed to further stratification into higher risk of recurrence groups for patient subpopulations initially featuring the usually more favorable low Gleason score tumor grades. See Veltri, et al., J Cell Biochem Suppl, (1994) 19:249-2583

Unfortunately, studies of HER-2/neu expression by IHC are subject to considerable technical variations. Given that most specimens are formalin fixed, paraffin-embedded archival material, false negative staining may occur due to antigen loss. Fixation and processing protocols significantly affect the reactivity of the antigenic determinants detected by HER-

2/neu antibodies such as MAB-1 and pAB-60. Ware, et al., Hum pathol, (1991) 22:254-258. Different antibodies may produce either cytoplasmic or membranous staining, be ineffective when certain fixatives are used or be impacted by temperature of the IHC reaction. Ware, et al., supra. Antigen retrieval techniques featuring either enzymatic digestion or microwave irradiation contribute additional potential variables that may affect staining levels. Potential sources of error in IHC studies of HER-2/neu oncogen expression in archival breast cancer tissue samples have recently been reported. See Press, et al., Cancer Res. (1994) 54:2771-2777. Substantial variation in sensitivity and specificity of commercially available HER-2/neu antibodies to detect gene amplification confirmed by Southern blotting was observed with antibodies such as the pAB-1, featuring 65% sensitivity and the 9G6, 47% sensitivity. Press, et al., supra. Fixation and embedding methods similarly affect the results of IHC for HER-2/neu protein in gastric cancer. See Chiu, et al., J Clin Pathol, (1994) 47:816-822. Staining interpretation problems and inter observer variability especially concerning cytoplasmic immunoreactivity for HER-2/neu protein have also been reported. See Kay, et al., J Clin Pathol, (1994) 47:816-822.

The present invention overscomes the above described problems associated with such variation in the immunohistochemical demonstration of HER-2/neu protein in archival tissue specimens. Fluorescence in-situ hybridization (FISH) has recently been employed in detectionof another chromosomal aneusomies and gene copy numbers in both solid tumors and hematopoietic malignancies. See, e.g., Woman SR., Pathology Annual, Appelton and Lang, Stanford, Conn., pp.227-244 (1995). Using chromosome specific probes, FISH was found to be more sensitive than flow cytometry for the detection of aneuploidy in prostate cancer. Visakorpi, et al., Am J Pathol, 145:624-630 (1994). High grade prostate cancer has been linked to chromosomal aneusomy by FISH and chromosome 8 aneusomy has been associated with increased tumor stage. Brown, et al., J Urol, 152:1157-1162 (1994). FISH detected aneusomy in prostate cancer has been associated with recurrent and progressive disease. See Lifson, et al., Anal Quant Cytol Histol,17:93-99 (1995); Koivisto, et al., Am J Pathol, 147:16-8-14 (1995); Lieber MM., J Cell Biochem (suppl), 19:246-248 (1994); Bandyk,m et al., Genes Chrom Cancer, 9:10-27 (1994); Zitzelsberger, et al., J Pathol, 172:325-335 (1994) Alcaraz, et al., Cancer Res, 54:3998-4002 (1994). Studies have revealed vaying abnormalities associated with disease progression including increased copy number of

chromosome X (Koivisto, et al., supra; Zitzelsberger, et al., supra; Alcaraz, et al., supra). FISH based techniques have also been utilized recently to demonstrate potential candidate tumor suppressor genes that may prove of significance in prostate cancer. Aao, et al., Am J Pathol, 147:896-904 (1995); Cher, J Urol, 153:249-254 (1995).

It has been hypothesized that the use of anti-androgens early in disease treatment, i.e. in a neoadjuvant approach prior to prostatectomy, hasten the development of the androgen-independent clones. Thus, a marker that could predict the "risk" that such a treatment with existing or future anti-androgen resistant tumors would be of significant clinical value.

SUMMARY OF THE INVENTION

In one aspect the invention relates to a method of determining the severity of prostatic cancer including measuring the level of amplification of the HER-2/neu gene in a sample of prostate tissue using fluorescence in-situ hybridization and comparing the measured level of amplification of the HER-2/neu gene in the sample with the level of HER-2/neu gene in normal prostate tissue.

In another aspect, the invention relates to a method for selecting treatment for prostate cancer including determining whether the number of copies of HER-2/neu gene in prostate cells from the patient exceeds four using fluorescence in-situ hybridization and aggressively treating such patients having prostate cells with five or more copies of the HER-2/neu gene.

In a related aspect, the invention relates to a method for selecting treatment for prostate cancer including determining whether the number of copies of HER-2/neu gene in prostate cells from the patient exceeds four using fluorescence in-situ hybridization and applying higher doses of radiation to the cancer patient whose cells contain five or more copies.

In yet another aspect, the invention relates to a method for selecting treatment for prostate cancer including determining whether the number of copies of HER-2/neu gene in prostate cells from the patient exceeds four using fluorescence in-situ hybridization and choosing a cancer cell specific treatment, such as Herceptin or bispecific antibody therapy for patients with cells in which Her-2/neu is amplified.

In another aspect, the invention relates to a method for treating prostate cancer by using compositions that block expression of the HER-2/neu oncogene or function of the gene

product. The HER-2/neu protein is a cell membrane tyrosine kinase that is a member of the epidermal growth factor receptor family. These compositions, which may include for example antibodies, vaccines, and gene therapy approaches, would be preferably employed in those tumors wherein the HER-2/neu gene is amplified. Similar therapeutic approaches have been successfully employed in battling breast cancer. See Drebin et al, Cell 42: 695-706 (1985), Drebin et al, Oncogene 2: 273-277 (1988), Drebin et al, Oncogene 2: 387-394 (1988) and Fendley et al, Cancer Research 50:1550-1558 (1990). However, it was heretofore unknown and unexpected that such agents could be used against prostate tumors. See Baselga et al, Journal of Clinical Oncology 14: 737-744 (1996), Cobleigh et al, Proc. ASCO 17:97a (1998) and Slamon et al, Proc. ASCO 17:98a (1998).

The reported data relating to prostate cancer and HER-2/neu has been extremely variable with a number of "negative" studies concerning prognostic significance. However, these studies have involved detecting HER-2/neu protein not the gene. See Vaiskorpi et al, Modern Pathology 5: 643-648 (1992), Ibriham et al, Surgical Oncology 1: 151-155 (1992), Kuhn et al, Journal of Urology 150: 1427-1433 (1993), Sadasivan et al, Journal of Urology 150: 125-131 (1993) and Ware et al, Human Pathology 22: 254-258 (1991).

In a related aspect, the invention relates to the combination of anti-androgen therapy and an inhibitor of HER-2/neu to treat prostate cancer. Such anti-androgen therapy could be, for example, Casodex™ (bicalutamide), Eulexin™ (flutamide), Lupron™ (leuprolide acetate), Zoladex™ (goserelin), estrogens, destruction or removal of androgen producing cells from the body, such as orchiectomy and combinations of these.

In a related aspect, the invention relates to a method for testing for HER-2/neu gene amplification and treating prostate cancer using anti-androgen therapy in patients which do not have the HER-2/neu gene amplified.

In another aspect, the invention relates to a method for selecting treatment for prostate cancer based on the determination that the number of copies of HER-2/neu gene in prostate cells from the patient. When the number of copies is abnormally high aggressive therapy is indicated and treatment with anti-androgen therapy is contraindicated unless combined with an inhibitor of HER-2/neu.

BRIEF DESCRIPTION OF THE DRAWING

FIGURE 1 is a Kaplan and Meier survival curve showing significant difference in disease recurrence for patients with prostate carcinoma featuring amplification of the HER-2/neu gene by fluorescence in situ hybridization compared with patients whose tumors were not amplified.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "prostate cancer" includes adenocarcinoma of the prostate, particularly when infiltrating the prostate stroma, prostatic epithelial neoplasia, and metastasis thereof regardless of their location.

An "anti-androgen" is an effective chemical or surgical treatment which reduces the amount of androgens in the blood, reduces the effect of androgens on prostate cells (especially prostate cancer) or acts on the cells to have an effect contrary or annulling the effect of an androgen. Examples of reducing blood concentrations include surgical removal (or destruction chemically or immunologically) of androgen producing cells such as orchiectomy or by addition of a composition. Examples of compositions include: estrogens such as diethyl stilbestrol, Lupron™ (leuprolide acetate) is a nonapeptide analog of naturally occurring gonadotropin releasing hormone (gn-RH or LHRH), thereby suppressing testicular steroidogenesis.by acting as an LHRH agonist and Zoladex™ (goserelin), a synthetic decapeptide analog of LHRH which leads to suppression of pituitary gonadotropins after sustained administration. It is believed to act as a potent inhibitor of pituitary gonadotropins upon sustained administration. Other techniques include removal with a hormone adsorbent or degrading enzyme or other agent. Examples of agents which reduce the effect of the androgen include Casodex™ (bicalutamide), which inhibits the action of androgens by binding to cytosol androgen receptors in the target tissue and Eulexin™ (flutamide) which inhibit androgen uptake and/or by inhibiting nuclear binding of androgen to a receptor in target tissues or both. Contrary effecting compositions include estrogens.

An "anti-HER-2/neu composition" includes compositions which act on the HER-2/neu DNA, the HER-2/neu mRNA (spliced or not), the HER-2/neu protein or inhibit or counter the activity of the HER-2/neu protein. Examples of a composition which acts on the DNA and RNA include anti-sense oligonucleotides or triple strand forming oligonucleotides which code for a DNA or RNA complementary to and capable of binding HER-2/neu mRNA or HER-

2/neu DNA, thereby preventing its transcription, splicing or translation into protein. Ribozymes may also be used which catalytically alter the HER-2/neu gene or mRNA. Examples of compositions which act on the protein include antibodies, fragments thereof, or other protein binding agents to the HER-2/neu protein; peptides which exhibit sufficient homology to the tyrosine kinase growth factor ligand to bind to and inactivate the HER-2/neu protein; antagonizing analogs to the HER-2/neu receptor; small molecule signal transduction inhibitors (Sugen, Inc.), a vaccine or other immunological preparations containing a chemical moiety resembling the HER-2/neu protein and capable of eliciting an immune response (antibodies or cellular immunity) against the HER-2/neu protein and enzymes which modify the protein by cleavage, altered glycosylation or altered three dimensional configuration. An example of a composition which counters the action of HER-2/neu is a drug with an antigrowth activity. Preferred compositions are à recombinant humanized monoclonal antibody such as Herceptin™ (Genentech, South San Francisco) and MDX-210 (Medarex), a bispecific antibody combination with the capability of directly linking the body's immune cells to the target cancer cells. A more complete description of Herceptin™ is found is found in Hudziak et al, U.S. Patent 5,725,856. Other antibodies to HER-2/neu and their uses are described above. Alternative treatments may include any therapeutic products designed to attack breast cancer cells expressing elevated levels or gene copy numbers of HER-2/neu. At the present time, no effective chemotherapy has been established. However, if chemotherapy were effective, it could also be administered in combination with, subsequent to or prior to the above treatment.

Recent studies demonstrated the correlation between HER-2/neu gene amplification and high Gleason grade and DNA aneuploidy and the ability to predict disease recurrence in patients with surgically treated prostate cancer using a method for detecting HER-2/neu gene amplification. Additionally, evidence exists of the prognostic value of HER-2/neu amplification in assessing the likelihood of disease recurrence in patients undergoing radiation therapy.

To detect HER-2/neu overexpression, one may assay for an excess amount of the HER-2/neu protein by immunoassay or other diagnostic protein assay such as gel electrophoresis. However, these techniques give results which are highly variable and do not measure the prognosis for prostate cancer as noted above. It may be possible to detect

overexpression of HER-2/neu by measuring HER-2/neu mRNA. However, RNA is easily degraded and difficult to quantitatively measure.

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In situ hybridization, especially fluorescence in-situ hybridization (FISH) is used in accordance with the present invention to detect amplification of HER-2/neu genes in prostate tissue and provide a reliable technique for assessing the prognosis of prostate cancer which is surprisingly more effective than the existing immunohistochemical (IHC) measurement of HER-2/neu protein overexpression. FISH detection of amplification of the HER-2/neu gene a prostate cancer tissue is compared herein with HER-2/neu protein expression as determined by IHC cellular proliferative activity as determined by immunohistochemical analysis of Ki-67 and correlated by logistic regression analysis with Gleason tumor grade, DNA ploidy, serum PSA and pathologic stage.

Normal cells contain 2 copies of each gene. After DNA replication and just before cell division, a cell may have 4 copies of a gene. The detection of five or more copies of the HER-2/neu gene clearly indicates the presence of amplified HER-2/neu genes. Identification of an amplified HER-2/neu status very early in the diagnostic process followed by treatment with anti-HER-2/neu treatment, such as a anti-HER-2/neu antibody-based compositions or a gene therapy which utilizes an anti-sense nucleic acid to inhibit HER-2/neu, can prevent the progression of the disease to more advanced stages. Additionally, patients who have an androgen-independent form of the disease may derive a clinical benefit from the administration of an anti-HER/2-neu treatment in combination with anti-androgen.

In accordance with the present invention increased copy number of the HER-2/neu gene in prostate tissues is detected using ISH techniques. The structure of the HER-2/neu gene is well known. See, e.g., King et al., Science, 229:974-978 (1985) and Coussens et al., Science, 230:1132-1139 (1986). Detectable DNA probes capable of hybridizing to the known HER-2/neu gene sequence are constructed and labeled using conventional techniques. See,

for example, PCT Application Pub. No. WO94/09022, the entire contents of which are incorporated herein by reference. Examples of labeling systems include those which incorporate digoxygenin, biotin, avidin, streptavidin and antibodies. Labeled DNA probes are then allowed to hybridize to available HER-2/neu genes and are detected using conventional fluorescence detecting techniques such as fluorescence microscopy, spectrophotometers, fluorescent plate readers and flow sorters. For signal detection, fluorescent molecules can be linked directly to the DNA probe or can be linked to a binding partner for the probe. Useful fluorescent molecules include, but are not limited to fluorescein, amino coumarin acetic acid, tetramethylrhodamine isothiocyanate, Texas Red, Cy3.0, Cy5.0, and green fluorescent protein. Other non-fluorescent labels may be used such as chemiluminescent, radioactive, enzyme, ligand, spin labels, quenchers etc., and the choice is well known and within the skill of the art. The selection among known labels in the DNA hybridization and other binding assays (e.g. immunoassay) Signal detection and amplification techniques known to those skilled in the art can be utilized in accordance with the present invention. Thus, signal detection and amplification techniques such as those involving streptavidin/biotin, avidin/biotin, hapten conjugates such as digoxigenin/anti-digoxigenin, dinitrophenyl and other known antibody based detection and amplification techniques are utilized herein.

Amplification of HER-2/neu correlates to a decreased chance of long term survival as well as a shortened time to relapse of the disease. See FIGURE 1. Determination of the HER-2/neu copy number in the prostate cells from an initial needle biopsy in accordance with the present invention can be used to identify patients with a biologically aggressive form of prostate cancer. The expected number of signals in a normal cell and in an unamplified tumor cell varies from 2 to 4 depending on the phase of the cell cycle. A cell with five or more signals is considered amplified. Individuals with cells in which amplification of the HER-2/neu gene is observed may require different or more aggressive treatment.

Conversely, patients having prostate cancer with a low copy number of HER-2/neu can be treated with milder conventional therapy, such an anti-androgen treatment alone, to lessen or avoid adverse side effects while containing the cancer or placed under observation thereby avoiding radiation or drug exposure entirely until such time as some therapeutic intervention is absolutely indicated.

In addition to traditional treatments such as surgical intervention, and higher doses of radiation, alternative methods of treatment for prostate and other cancers are being developed. Alternative treatments may include therapeutic products designed to attack specific cancer cells. Specifically, compositions directed against cancers which exhibit overexpression of the HER-2/neu protein would be desirable. Such compositions include antibodies, or fragments thereof, to the HER-2/neu protein and peptides which exhibit sufficient homology to the tyrosine kinase growth factor ligand. These compositions may be linked to a marker moiety, which is readily recognized as foreign by the patient, and cytotoxic moieties (e.g. ricin chain) or structures (liposomes, etc. containing a drug).

Prostate cancer is known to be androgen sensitive and generally responds to treatment that counteracts the effect of androgen and/or removes the source of androgen in many cases. Castrate levels of testosterone can be achieved with surgical orchiectomy or by administration of a an anti-androgen. An anti-androgen is any substance which inhibits the synthesis or action of androgen. There are several anti-androgenic compounds currently in clinical use. Diethylstilbestrol (DES), an estrogenic compound providing therapeutic responses similar to natural estrogens, Casodex™ (bicalutamide), for example, inhibits the action of androgens by binding to cytosol androgen receptors in the target tissue. Eulexin™ (flutamide 750 mg/day orally in three dosages) exerts its anti-androgenic action by inhibiting androgen uptake and/or by inhibiting nuclear binding of androgen in target tissues or both. Lupron™ (leuprolide acetate 1 mg/day by injection) is a nonapeptide analog of naturally occurring gonadotropin releasing hormone (gn-RH or LHRH), and, therefore, acts as an LHRH agonist suppressing ovarian and testicular steroidogenesis. Similarly, Zoladex™ (goserelin), a synthetic decapeptide analog of LHRH acts as a potent inhibitor of pituitary gonadotropins. Sustained administration of Zoladex™ leads to suppression of pituitary gonadotropins; as a result, serum levels of testosterone fall into the range normally seen with surgical castration 2-4 weeks after initiation of therapy.

Unfortunately, some prostate carcinomas eventually become refractory in the presence of anti-androgen, that is, their growth becomes androgen-independent. The HER-2/neu gene amplification rate for men with progressive hormone refractory disease was about 67%, nearly twice the rate of amplification seen in the non-hormone refractory group.

Thus, a composition which acts on cells having amplified HER-2/neu compensates for a weakness in anti-androgen therapy, namely resistance related to amplified HER-2/neu. Combination therapy acts on the prostate cancer cells from two opposing mechanisms of action. Cells resistant to one therapy are believed to do so by mutating or being selected to be susceptible to the other therapy. Thus, their use in combination is desirable, particularly in view of the prognosis for prostate cancer.

While advanced prostate cancers are typically the most demanding in treatment effectiveness, the treatments of the present invention may also be used for early stage prostate cancers. Since earlier treatment is generally more successful than when the patient is terminal, early treatment, even at the point of initial diagnosis is within the present invention.

Anti-HER-2/neu and anti-androgen compositions used are pharmaceuticals (biologicals, e.g. vaccines, are considered pharmaceuticals) and typically are mixed with a vehicle or carrier and which are pharmaceutically acceptable. The nature of the pharmaceutically acceptable carrier or vehicle, its selection and formulation based on active ingredient and route of administration is well known to those skilled in the art.

The two compositions may be mixed in the same container, unitary dosage or they be in separate containers. In either situation, a kit may be formed containing one or more of the compositions along with instructions for usage treating prostate cancer. The kit may be in a number of different configurations such as one or more containers in a single box or other manner linking the two compositions in close proximity to each other. Also, the linkage may be indirect by way of the instructions contained in packages of one or both drugs.

The following examples are included for purposes of illustrating certain aspects of the invention and should not be construed as limiting.

EXAMPLE 1: HER-2/neu GENE COPY NUMBER AND POST-SURGICAL DISEASE RECURRENCE

One hundred thirteen men ranging in ages from 49-88 years with a mean of 66 years how were diagnosed with prostatic adenocarcinoma and underwent radical retropubic prostatectomy between 1987 and 1996 were randomly selected from surgical pathology files. The microscopic slides from each case were reviewed and the tumors were graded and staged according to the Gleason (See Gleason, Human Pathology, 23:273-279 (1992)) and TNM

(Beahrs et al, Manual for Staging of Cancer by American Joint Committee on Cancer, J.B. Lippincott Co., (1992)) systems respectively. The pre-operative serum PSA (Tandem method, Hybritech) was obtained from a review of the medical records in all cases. The preoperative serum prostatic specific antigen levels ranged from 0.8 ng/ml to 87.8 ng/ml with a mean of 12.1 ng/ml. There was no correlation between pre-operative serum PSA level and any of the other prognostic variables or disease outcome. The mean clinical follow-up was 42 months (range 4 to 106 months). Disease recurrence was defined as a post-operative serum PSA level equal to or greater than 0.4 ng/ml.

When divided into two groups consisting of low grade cases with Gleason score six or lower (58 cases) and high grade cases with Gleason score seven or higher (55 cases), tumor grade correlated with post-operative disease recurrence (p=0.013) (Table 1). When divided into three groups consisting of low grade Gleason score 2-5; intermediate grade Gleason score 6 & 7; and high grade Gleason score 8-10, similar significant correlation of grade with disease outcome was observed on univariant analysis (p=0.0001).

EXAMPLE 2: FISH ASSAY FOR HER-2/NEU

The assay was performed by the Inform® HER-2/neu Gene Detection System (FDA approved version). Briefly, unstained four micron formalin-fixed paraffin-embedded tissue sections were applied to silanized slides and processed according to the Oncor chromosome in-situ hybridization system (Oncor, Inc., Gaithersburg, MD). Briefly, tissue de-paraffinization in xylene was followed by transfer through two changes of 100% ethanol and the slides were allowed to air dry. The slides were then immersed for 30 minutes in 30% Oncor pretreatment solution (30% sodium bisulfite in 2x SSC (0.45 molar NaCl and 0.045 molar sodium citrate)) at 45°C and 45 minutes in Oncor protein digesting solution (0.25 mg/ml proteinase K in 2x SSC) at 45°C. After a brief wash in 2X sodium chloride/sodium citrate (SSC) slides were dehydrated in 100% ethanol and allowed to air dry. Oncor unique sequence digoxigenin-labeled HER-2/neu DNA probe consisting of 4 contiguous overlapping cosmid probes which create a 90 kb unbroken DNA strand (available from Oncor, Inc. Catalog Nos. P5111-BIO, P5111-DIG, P5111-B.5, P5111-DG.5, S8000-KIT or S8000-KIT-E) was prewarmed for five minutes at 37°C prior to application. The amount of probe hybridization mixture was approximated according to the target area and the size of the

coverslip to be placed over the tissue during hybridization (10 µl probe mixture per 22 x 22 mm coverslip area). Denaturation was accomplished at 69°C for five minutes and the slides were then incubated overnight at 37°C in a pre-warmed humidified chamber. Following overnight hybridization slides were again immersed in 2X SSC and pre-warmed to 72°C for a five minute stringency wash in 40 ml 2X SSC at pH 7.0 prior to detection. Fluorescein-labeled anti-digoxigenin (commercially available from Boerhinger Mannheim) in a solution containing 5% nonfat dry bovine milk, 0.08% sodium azide, 0.05% NP-40, 0.1 molar NaH_2PO_4 and 0.1 molar $K_2H_2PO_4$ was applied and a plastic coverslip placed gently for a 20 minute incubation at 37°C in a pre-warmed humidified chamber in the dark. After careful removal of the coverslip and rinsing of excess detection compounds in 1X phosphate-buffered detergent (PBD) for three rinses at two minutes each, slides were counterstained with 18µl of propidium iodide/antifade (1:4) and covered with a glass coverslip. Slides were evaluated for HER-2/neu gene copy number using a Zeiss Axioskop 50 fluorescence microscope.

The probe displays a single fluorescent signal at the location of each copy of the HER-2/neu gene. The expected number of signals in a normal cell and in an unamplified tumor cell varies from 2-4 depending on the phase of the cell cycle. A cell with five or more signals was considered amplified. A minimum of 100 tumor cells in each prostate carcinoma specimen was evaluated for the number of nuclear HER-2/neu signals. Amplified tumors were defined as having a minimum of 20 cells with five signals or greater per cell. The number of signals was not averaged between cells.

Forty-one percent of the prostate cancers featured amplification of the HER-2/neu gene by FISH (Table 1). Tumors with gene amplification generally featured greater than 8 individual signals per nucleus in the adenocarcinomas which contrasted with the average of signals per nucleus in the adjacent benign prostate tissue and stromal elements. Virtually all the nuclei shown in a poorly differentiated high grade four micron paraffin-embedded formalin fixed prostate cancer tissue section, reveal fluorescence signals of HER-2/neu hybridization that are almost too numerous to count. Amplification of HER-2/neu gene by FSH significantly correlated with high tumor grade ($p=0.029$). In patients with prostate cancer featuring HER-2/neu gene amplification by FISH, the disease was 2.3 times more likely to recur than in patients whose tumors did not feature HER-2/neu amplification. HER-2/neu gene amplification by FISH was identified in 27% of pathologic stage 2 tumors whereas

pathologic stage 3 and 4 tumors featured a 59% amplification rate. This association reached near significance on univariate analysis (p=0.06). There was no correlation of HER-2/neu amplification by FISH with the pre-operative serum PSA level.

DNA ploidy Analysis

A five micron thick tissue section from the formalin-fixed paraffin-embedded tumor tissue was stained by the Feulgen method and evaluated for total DNA content using the CAS 200 Image Analyzer (Becton Dickinson Cellular Imaging Systems, Mountainview, CA) as previously described. Fournier, et al., supra. A DNA index of greater than 1.23 was considered non-diploid (aneuploid). Tetraploid peaks greater than 15% of the total cell population were considered non-diploid. Tetraploid peaks equal to or less than 15% of the total cell population were considered non-diploid. Tetraploid peaks equal to or less than 15% of the total cell population were considered to be the G_2M components of diploid cell populations.

When divided into two groups of 69% (61%) diploid cases and 44 (39%) non-diploid cases, the presence of non-diploid DNA content correlated with post operative disease recurrence on univariate analysis (p=0.016). DNA content correlated with tumor grade with 39 of 44 (89%) of the non-diploid tumors featuring high tumor grade (p=0.001).

Immunohistochemistry

Unstained five micron sections of formalin-fixed paraffin embedded tissue samples were deparaffinized, rehydrated and immersed in preheated 10mM citrate buffer, pH 6.0. Slides were boiled at high power in a microwave oven for 15 minutes and allowed to stand for 30 minutes at room temperature. The slides were stained on the Ventana ES Automated Immunohistochemistry System (Ventana Medical Systems, Tucson, AZ) employing the Ventana indirect biotin avidin DAB detection system. Endogenous peroxidase was blocked and sections were incubated for 32 minutes at 41°C with rabbit anti-human c-erb-B2 (HER-2/neu) at a 1:40 dilution (Dako Corp., Carpenteria, CA). Following primary antibody incubation, slides were sequentially incubated with universal biotinylated immunoglobulin secondary antibody, avidin horseradish peroxidase conjugate and diaminobenzide (DAB) followed by copper sulfate enhancement. Slides were counterstained with hematoxylin.

Negative control slides were included to establish background and non-specific staining of the primary and secondary antibodies and/or detection kit reagents.

A breast cancer specimen known to be positive for HER-2/neu protein expression was utilized as a positive control. Only those cases in which a majority of the tumor cells showed either an intense cytoplasmic and/or diffuse membranous staining were considered positive. Cases that were judged negative included complete lack of immunoreactivity and weak or focal staining patterns.

By IHC, 29% of the prostate cancers featured intense cytoplasmic or diffuse membranous immunoreactivity indicative of $p185^{neu}$ overexpression (FIG. 3). Protein overexpressed by IHC correlated with tumor grade ($p=0.03$), but not with ploidy ($p=0.125$). A trend for protein overexpression by IHC and gene amplification by FISH in the same prostate cancer specimen did not reach statistical significance ($p=0.25$). In addition, HER-2/neu protein overexpression by IHC did not predict post-operative disease recurrence (Table 1).

The correlation of HER-2/neu protein expression by IHC and gene amplification status by FISH with tumor grade, DNA ploidy, pathologic stage and pre-operative serum PSA was performed using the Chi square model. A p value of less than 0.05 was considered significant. Univariate and multivariate analysis for the prediction of pathologic stage and post-operative disease recurrence by tumor grade, DNA ploidy, IHC and FISH was performed using the Cox proportional hazards model. A p value of less than 0.05 was considered significant. The impact of each prognostic variable on disease recurrence was also studied using the method of Kaplan and Meier. FIGURE 1 depicts the results for HER-2/neu amplification.

When stratified into groups of PSA levels less than 10 ng/ml and PSA levels equal or greater than 10 ng/ml, there was no significant correlation of serum PSA with disease recurrence. When stratified into two pathologic stage groups of stage 2 (36% of patients) and stage 3 and 4 (64% of patients), no correlation of pathologic stage with subsequent disease recurrence was found.

On multivariate analysis using the Cox regression model tumor grade (=0.0001) were independent outcome predictors. The prognostic value of HER-2/neu amplification by FISH in the prediction of post-operative disease recurrence on univariate analysis ($p=0.029$) was

reduced on multivariate analysis by either tumor grade or DNA ploidy status to near independent significance (p=0.125).

Significant association of HER-2/neu gene amplification with tumor grade and DNA ploidy and correlation with disease recurrence after radical prostatectomy is shown. Tumor grade and DNA ploidy status were independent predictors of outcome. The prognostic value of HER-2/neu gene amplification by FISH reached near independence on multivariate analysis being reduced by either grade or ploidy status. This data shows that HER-2/neu gene amplification by FISH is of significant value in predicting disease outcome, while use of IHC to detect HER-2/neu protein overexpression did not predict post-operative disease occurrence (Table 1).

TABLE 1

PROGNOSTIC MARKER	RISK FACTOR	% of Cases at Risk	Significant Correlation With Disease Recurrence	
			Univariant	Multi-Variant
Pre-operative PSA	10 ng/ml or higher	19%	no	no
Pathologic Stage	Stage 3 or Stage 4	64%	no	no
Tumor Grade	Gleason 7 or higher	49%	yes	yes
DNA Ploidy	non-diploid	39%	yes	yes
HER-2/neu Amplification by FISH	Amplified	41%	yes	no*
HER-2/neu Overexpression by IHC	Overexpressed	29%	no	no

* Independent status of HER-2/neu amplification by FISH reduced by either grade or ploidy status to near significance (p=0.129).

EXAMPLE III HER-2/neu COPY NUMBER AND PATIENT RESPONSE TO RADIATION THERAPY

Radiation therapy can have varying outcomes depending on disease stage. Survival rates for patients with stage B disease are 83% at 5 years and 66% at 10 years. For patients with stage C disease, the rates are 76% and 46%. Respectively, While these survival rates are significant, the problem of disease recurrence in the form of local failure or metastasis to other tissues remains.

It has been reported that higher doses of radiation substantially decrease the local failure rate (LFR). Increasing the dose from 59 to 60 Gy, for example reduced the LFR from 24% to 13% in patients with stage B disease. A similar disease, from a LFR of 25% to 17% was seen in patients with stage C disease when radiation dose was increased from less than 70 Gy to greater than 70 Gy.

Amplification of the HER-2/neu gene in prostate cancer patients has been shown to be of value in predicting post-radiation recurrence of the disease.

Forty-two men ranging in age from 54 to 77 years with a mean of 69 years who were diagnosed with prostatic adenocarcinoma and who underwent radiation therapy between 1989 and 1995 were randomly selected from surgical pathology and radiation oncology files. As in Example 2, the pretreatment slide for each patient was examined and the tumor graded by assigning Gleason scores. The pretreatment serum level of PSA for each patient was obtained from the file. Pretreatment serum levels of PSA ranged from 1.3 to 100 ng/mL with a mean of 16.2 ng/mL.

When the post-radiation serum level of PSA rose to greater than 0.5 ng/mL on two consecutive measurements, the radiation therapy was viewed as ineffectual and the patient was considered to have biochemical evidence of disease recurrence.

When divided in two groups consisting of low grade cases with Gleason score six or lower (23 cases) and high grade cases with Gleason score seven or higher (19 cases), there was no correlation with initial biopsy tumor grade and post-radiation disease recurrence.

An immunochemically method such as the one described in Example 2 was employed to assess cellular proliferative activity in prostate cells in pretreatment biopsy specimens based on the binding of MIB-1. MIB-1 is an anti-human monoclonal antibody reactive to Ki-67

antigen, a nuclear protein complex expressed when the cell leaves the quiescent phase of the cell cycle (G_0).

Evaluation of the staining was conducted by three observers jointly. A cell proliferation index (CI) was obtained for each tumor based on the mean number of positively stained nuclei per high power field (HPF) (400 X magnification). A mean of five or more MIB-1 positive cells per HPF wee designated as high CPI cases; low CPI cases were those with a mean of four or less. Fifty-seven percent (24) of the tumors had a high CPI. Eighteen of the 23 tumors from patients with disease recurrence, (78%) were in the high CPI category, while only 32% of tumors from patients with no recurrence fell into the high CPI group.

Specimens were also evaluated for HER-2/neu gene amplification status using the FISH method described in Example 2. The results are shown in Table 2.

Using the criterion of a minimum of 20 malignant cells having counts of five signals or greater, 17 cases, that is, forty percent demonstrated amplification of HER-2/neu gene by FISH (Table 2). Of the 23 tumors that recurred, 12 (52%) exhibited HER-2/neu amplification. Twelve of the 17 cases featuring HER-2/neu amplification (71%), therefore, experienced post-radiation recurrence of the disease.

TABLE 2

MIB-1 and HER-2/neu Profile in Irradiated Prostatic Carcinomas

		Recurrence Status		
Parameter	Staining Results, Total Cases, 42%	PRR Total Cases 23%	No PRR Total cases, 19%	P Value
B-1 (high cell proliferation index)	24/32 (57)	18/23 (78)	6/19 (32)	0.007
HER-2/neu (gene amplification)	27/42 (40)	12/23 (52)	5/19 (26)	0.08

EXAMPLE IV: HER-2/NEU GENE COPY NUMBER AND TREATMENT WITH ANTI-ANDROGENS

To investigate the relationship between HER-2/neu gene amplification and androgen-independent prostate cancer, prostate tissue specimens from patients by needle biopsy, or other tissue removal, who had undergone hormone therapy were examined. In the group that received hormone therapy, 50 mg Casodex™ (bicalutamide) was administered daily to patients in combination with an LHRH agonist Eulexin™ (flutamide 750 mg/day orally) to maintain androgen at castrate levels. Patients in a refractory group who experienced progression of the prostate cancer in the absence of hormone treatment were evaluated as well. Serum PSA levels and bone pain index were used as indicators of progression of the disease.

From the cohort, 220 men with prostate cancer were selected. All of the men had surgery and the removed tumor tissue was assayed for number of copies of the HER-2/neu gene. HER-2/neu gene amplification was determined by FISH, as in Example 2. All men were placed on anti-androgen therapy of 50 mg Casodex™ (bicalutamide) daily.

From the cohort, 20 of the men with prostate cancer had a rising PSA level on this anti-androgen therapy. These patients are considered non-responders. HER-2/neu was amplified in the primary tumor of 14 (70%) of these men.

200 men with prostate cancer have a stable PSA level on this anti-androgen therapy. These patients are considered responders. This group included both "cured" and relapse patients whose cancer remains sensitive to anti-androgen therapy. HER-2/neu was amplified in the primary tumor of 35% of these men.

EXAMPLE V: HER-2/neu COPY NUMBER AND PATIENT THERAPY RESPONSE TO RECOMBINANT HUMANIZED MONOCLONAL ANTIBODY TO HER-2/neu

To evaluate the effectiveness of recombinant humanized monoclonal antibody to HER-2/neu treatment in older prostate cancer patients with advanced stage disease, disease progression despite treatment with anti-androgens, and no history of other malignancy, except non-melanoma skin cancer, the following study is performed.

To participate in the study, patients must demonstrate progression of adenocarcinoma of the prostate, while on the primary anti-androgen hormone therapy. Progressive disease is

defined by evidence of new osseous lesions as detected by bone scan. Alternatively, evidence of a greater than 25% increase in bidimensionally measurable soft tissue disease constitutes progression. An increasing serum PSA despite maintenance of castrate levels of testosterone also is indicative of progression of the disease. Additionally, to rule out patients experiencing an improvement due to anti-androgen withdrawal, patients in the study were required to show progression of the disease and be off of the anti-androgen therapy for at least four weeks prior to enrollment.

Biopsy or surgically removed tumor cells are assayed for HER-2/neu amplification using the technique of Example 2.

Patients receive 4 mg/kg Herceptin™ (Genentech, Inc., South San Francisco, CA) administered IV over 90 minutes, as a loading dose on Day 0. Subsequently, 2 mg/kg is administered IV over 30 minutes weekly for up to 24 weeks, or until disease progression or unacceptable side effects necessitated removal of the patient from the study.

The results of this study indicate whether treatment with Herceptin™ bestows a clinical benefit by slowing or halting the progression of the prostate cancer. Additionally, administration of Herceptin™ as a single agent confers a survival advantage in prostate cancer patients whose prostate cells from the initial needle biopsy demonstrated HER-2/neu amplification as compared to patients without HER-2/neu amplification.

Early intervention by identifying the prostate cancer patient at the time of the initial needle biopsy as being HER-2/neu amplified and beginning Herceptin™ treatment is expected to enhance the patients chances of survival by slowing or stopping progression of the disease while avoiding treatment in individuals who will not significantly benefit from Herceptin™ treatment.

EXAMPLE VI: TREATMENT OF PROSTATE CANCER WITH ANTI-HER-2/neu AND ANTI-ANDROGEN

The techniques of Example V are repeated on another group of 15 prostate cancer patients filling the same criteria. In addition to Herceptin™ treatment, the patients are simultaneously treated with anti-androgen therapy of 50 mg Casodex™ (bicalutamide), and Eulexin™ (flutamide 750 mg/day orally) daily.

The results of this study indicate whether combined treatment with an anti-HER-2/neu composition and an anti-androgen treatment bestows a clinical benefit by slowing or halting the progression of the prostate cancer over Herceptin™ as a single agent.

The effectiveness of this treatment indicates that earlier treatment of prostate cancer at the time of the initial needle biopsy, indicating HER-2/neu amplified, with an anti-HER-2/neu treatment and an anti-androgen treatment is expected to enhances the patients chances of survival by slowing or stopping progression of the disease.

EXAMPLE VII: TREATMENT OF PROSTATE CANCER WITH A HER-2/NEU VACCINE WITH OPTIONAL ANTI-ANDROGEN TREATMENT

The techniques of Example IV are repeated on another group of 25 prostate cancer patients filling the same criteria. However instead of receiving Herceptin™ treatment, the patients are vaccinated with a HER-2/neu vaccine previously proposed for treating breast cancer. 0.2 ml of this vaccine contains about 1 mg HER-2/neu protein previously produced by recombinant NIH 3T3 cells containing an expression vector with the cloned HER-2/neu gene. 10 of the patients simultaneously receive the anti-androgen therapy of 50 mg Casodex™ (bicalutamide) and Eulexin™ (flutamide 750 mg/day orally) daily.

Response is quantified by measuring serum PSA levels weekly for at least 12 weeks. While not actually used in this Example for technical reasons, the formation of anti-HER-2/neu antibody and/or cellular immune response in the patient's serum may be measured, correlated to all other medical data and used as a predictor of prognosis or determination of further treatment also.

The results of this study indicate whether the vaccine alone or combined with an anti-androgen treatment bestows a clinical benefit by slowing or halting the progression of the prostate cancer.

The effectiveness of this treatment indicates that earlier treatment of prostate cancer at the time of the initial needle biopsy, indicating HER-2/neu amplified, with a vaccine to HER-2/neu alone or with an anti-androgen treatment is expected to enhance the patients chances of survival by slowing or stopping progression of the disease.

It will be understood that various modifications may be made to the embodiments disclosed herein. Therefore, the above description should not be construed as limiting, but

merely as exemplifications of preferred embodiments. Those skilled in the art will envision other modifications within the scope and spirit of the claims appended hereto. All patents and references cited herein are explicitly incorporated by reference in their entirety.

What is claimed is:

1. A method for selecting a treatment for prostate cancer comprising:

(a) measuring the level of amplification of the HER-2/neu gene in a sample of prostate tissue using in-situ hybridization;

(b) determining whether the HER-2/neu gene copy number exceeds 4 copies per cell; and

(c) choosing a prostate cancer treatment wherein the treatment is chosen from the group consisting of higher dose radiation, tumor excision, and treatment with an anti-HER-2/neu composition when the number of copies of the HER-2/neu gene per cell exceeds four and the treatment is an anti-androgen or observation only when the number of copies of the HER-2/neu gene per cell is four or less.

2. A method for selecting a treatment for prostate cancer according to claim 1 wherein the treatment is applying radiation to the patient when the number of copies of the HER-2/neu gene per cell exceeds four.

3. A method for selecting a treatment for prostate cancer according to claim 2 wherein the radiation dose is in range of 60-75 Gy.

4. A method for selecting a treatment for prostate cancer according to claim 2 wherein the radiation dose is in the range of 70-75 Gy.

5. A method for selecting a treatment for prostate cancer according to claim 1 wherein treatment is administering an anti-HER-2/neu composition to the patient when the number of copies of the HER-2/neu gene per cell exceeds four.

6. A method for selecting a treatment for prostate cancer according to claim 1 wherein treatment is surgically excising the tumor from the patient when the number of copies of the HER-2/neu gene per cell exceeds four.

7. A method for selecting a treatment for prostate cancer according to claim 1 wherein treatment is an anti-androgen or observation only when the number of copies of the HER-2/neu gene per cell is four or less.

8. A method for treating prostate cancer comprising: administering to a patient in need of such treatment a therapeutically effective amount of an anti-HER-2/neu composition.

9. The method of claim 8 wherein said anti-HER-2/neu composition comprises an antibody or fragment thereof.

10. The method of claim 9 wherein said anti-HER-2/neu antibody is a recombinant humanized monoclonal antibody.

11. The method of claim 8 wherein said anti-HER-2/neu composition comprises a peptide or protein.

12. The method of claim 8 wherein the patient has a HER-2/neu gene copy number exceeding 4 copies per cell.

13. The method of claim 8 wherein said anti-HER-2/neu composition comprises an oligonucleotide.

14. The method of claim 8 further comprising administering an effective anti-androgen therapy to the patient.

15. The method of claim 8 wherein the prostate cancer is an androgen-independent prostate cancer.

16. The method of claim 14 wherein the anti-androgen exerts its anti-androgenic action by inhibiting androgen receptors or by inhibiting production of hormones.

17. The method of claim 16 wherein the anti-androgen is selected from the group consisting of flutamide, leuprolide acetate, bicalutamide or goserelin.

18. The method of claim 11 wherein the anti-HER-2/neu composition is a vaccine.

19. A composition comprising (a) an anti-HER-2/neu composition and (b) an anti-androgen.

20. The composition according to claim 19 wherein the anti-HER-2/neu composition is a recombinant humanized monoclonal antibody.

21. A therapeutic kit comprising: (a) a container containing at least one dose of an anti-HER-2/neu composition and (b) a container containing at least one dose of an anti-androgen, wherein said dose is an effective amount for treating prostate cancer.

22. The kit according to claim 19 wherein the anti-HER-2/neu composition is a recombinant humanized monoclonal antibody.

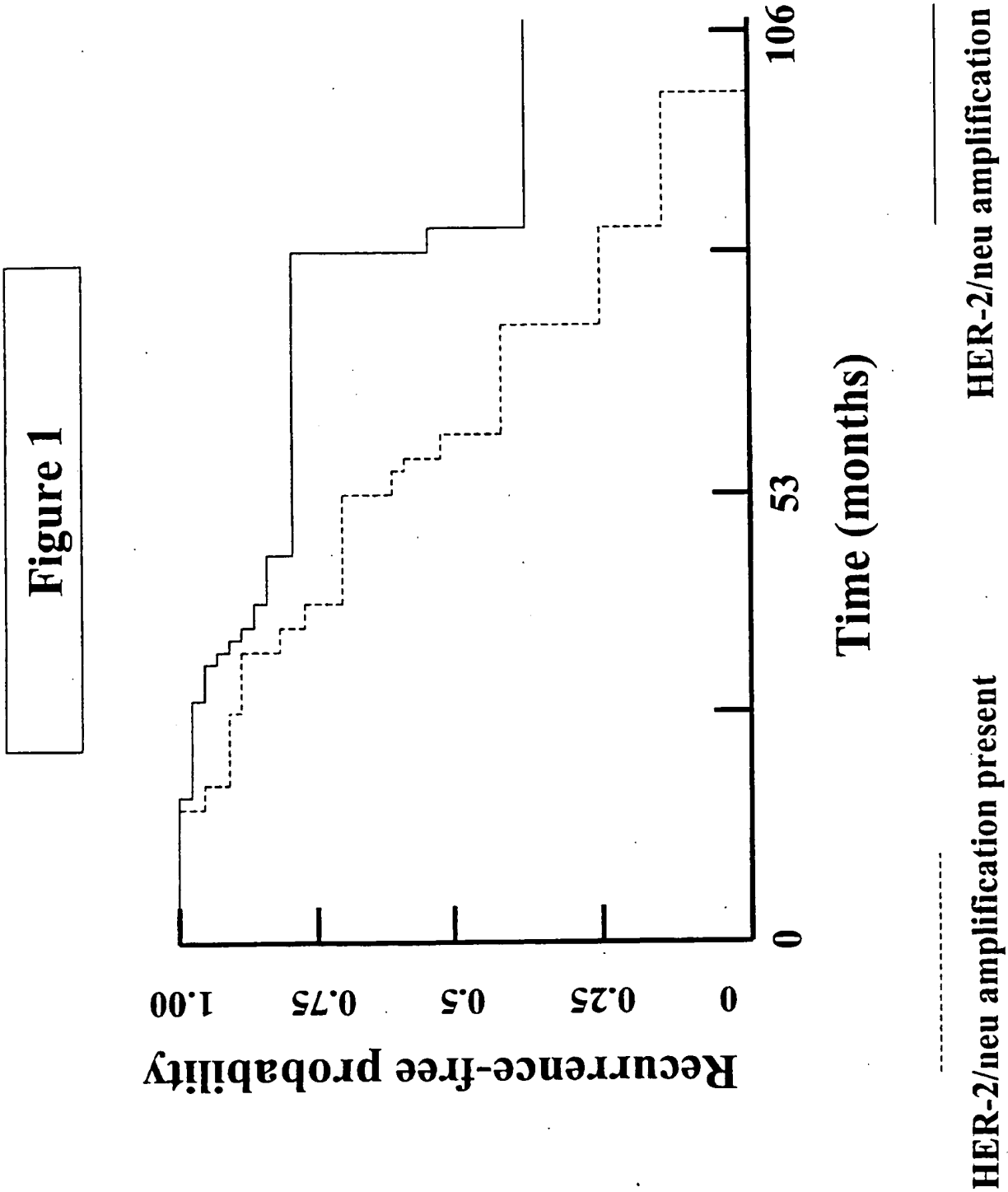
23. A method for determining therapy for a patient with prostate cancer comprising determining whether cells of the prostate cancer contain an abnormally elevated number of copies of HER-2/neu gene wherein an abnormally elevated number indicates therapy with an anti-HER-2/neu composition and a normal number indicates no therapy with an anti-HER-2/neu composition.

24. The method of claim 23 wherein the abnormally elevated number is at least 5.

25. The method of claim 23 wherein a normal number indicates therapy with an anti-androgen.

26. The method of claim 23 wherein an abnormally elevated number indicates therapy with both an anti-HER-2/neu composition and an anti-androgen.

Figure 1


Recurrence-free probability
0
0.25
0.5
0.75
1.00
0
53
106
Time (months)
HER-2/neu amplification present
HER-2/neu amplification absent

INTERNATIONAL SEARCH REPORT

Intern. ,nal application No.

PCT/US99/21153

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/68; C12P 19/34; C07H 21/02, 21/04; A61K 48/00, 39/395, 39/00;

US CL : 435/6, 91.2; 536/23.1, 24.3, 24.5; 514/44; 530/350, 387.1, 388.1; 424/85.8, 88

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system foll.wed by classification symbols)

U.S. : 435/6, 91.2; 536/23.1, 24.3, 24.5; 514/44; 530/350, 387.1, 388.1; 424/85.8, 88

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DESCOTES et al. Human Breast Cancer: Correlation study between HER-2/neu amplification and prognostic factors in an unselected population. Anticancer Res. 1994, Vol. 13, pages 119-124, see entire document.	1-26
Y	NARAGHI et al. Human prostate cancer overexpression of ErbB2 may be due to HER2/neu gene amplification. Proceedings American Association for Cancer Research. March 1995, Vol. 36, Abstract A3838, page 645, see entire document.	1-26
Y	MYERS et al. Serum levels of erbB-2 protein in prostate adenocarcinoma. Proceedings American Association for Cancer Research. March 1995, Vol. 36, Abstract A3838, page 645, see entire document.	1-26
Y	PATERSON et al. Correlation between c-erbB-2 amplification and risk of recurrent disease in node-negative breast cancer. Cancer Res. 15 January 1991, Vol. 51, pages 556-567, see entire document.	1-26
Y	HANKS et al. Optimization of conformal radiation treatment of prostate cancer: report of a dose escalation study. Int. J. Radiation Oncology Biol. Phys. February 1997, Vol. 37, No. 3, pages 543-550, see entire document.	1-26
Y	ROSS et al. HER-2/neu oncogene amplification and p34CDC2 cylcin dependent kinase overexpression product prodict recurrence in prostate cancer. Proc. American Association Cancer Research. March 1997, Vol. 38, page 277, see entire document.	1-26

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 December 1999 (08.12.1999)	14 FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Gary Jones Telephone No. 703-308-1196

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INTERNATIONAL SEARCH REPORT

Inter ional application No.

PCT/US99/21153

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FERNANDEZ-TRIGO et al. Prognostic implications of chemoresistance-sensitivity assays for colorectal and appendiceal cancer. Am. J. Clin. Oncol. October 1995, Vol. 18, No. 5, pages 454-60, see entire document.	1-26
A	PROBST et al. The G-tetrad in antisense targeting. Trends in Genetics. August 1996, Vol. 12, No. 8, pages 90-91, see entire document.	13
A	HARRIS et al. Strategies for targeted gene therapy. Trends in Genetics. October 1996, Vol. 12, No. 10, pages 400-405, see entire document.	13
A	MARSHALL. E. Gene Therapy's growing pains. Science. 15 Au 1995, Vol. 269, pages 1050-1055, see entire document.	13

INTERNATIONAL SEARCH REPORT

Int. tional application No.

PCT/US99/21153

Continuation of B. FIELDS SEARCHED Item 3: WEST, MEDLINE, BIOSIS, CAPLUS, JICST-EPLUS
search terms: Her2, neu, cancer, prostate, breast, tumor, four, copies, androgen, antibody, antisense, monoclonal, flutamide, goserelin, leuprolide, bicalutamide, herceptin